**[P-P&F.71]**

# High Cytotoxic Activity of a Bifunctional Chimeric Protein Containing a Ribosome Inactivating Protein (RIP) and a Serine Protease Inhibitor (WSCI)

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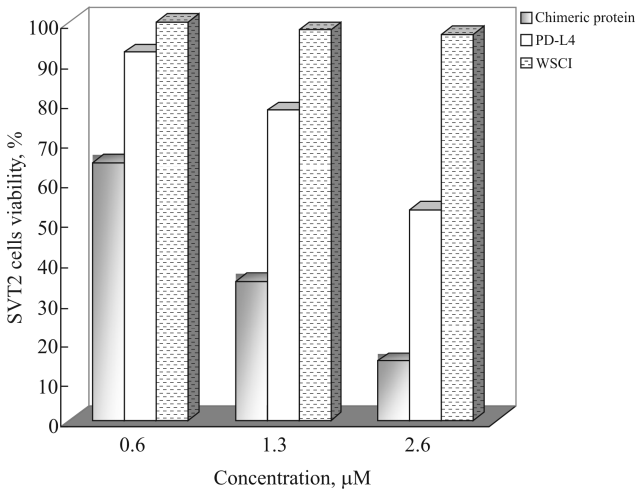
*Introduction:* Plants have their own networks of defence tools to protect against pathogens; these tools include a vast array of proteins: pathogenesis-related proteins, defensins, ribosome-inactivating proteins, lipid-transfer proteins, killer proteins, protease inhibitors, etc. (1). Recombinant DNA technology is currently used in agriculture to create genetically modified plants with an increased resistance to phytopathogens. In order to provide a more effective control of phytophagous insects, a bifunctional chimeric protein, potentially able to act as insecticide, has been designed and expressed in *E. coli* cells. The N-terminal domain corresponds to the toxic/antiviral protein PD-L4 type 1 RIP, firstly isolated from *P. dioica* L. leaves (2). The second domain corresponds to the wheat inhibitor WSCI, which is able to interfere with digestive proteases of mammals and insects (3, 4).

*Materials and methods:* The chimeric construct, *pd-l4-cDNA-oligonucleotide linker- wsci-cDNA*, was cloned in the expression vector pET22b and employed in transforming *E. coli* (strain BL21-DE3) (5). Cytotoxicity assays were carried out using Simian-virus-40-transformed mouse fibroblasts (SVT2 cells). The cells were plated at a density of $2.5\times10^3$ cells per well in 100 µL of medium. The cytotoxicity was determined in presence of increasing concentrations of PD-L4, WSCI and PD-L4/WSCI chimera. Cell survival was determined after 72 hours by means of MTT reduction assay.

*Results and discussion:* The protein chimera PD-L4/WSCI was expressed in *E. coli* and recovered from the inclusion bodies. Both tandem domains (PD-L4 and WSCI) retained their original activities (2, 3). Characterization of the chimera was performed by electrophoretic, chromatographic and N-terminal sequence analyses. Cytotoxicity assays revealed that the chimeric protein strongly affected the viability of SVT2 cells; surprisingly, the recorded levels of toxicity were greater than those observed for PD-L4 (see figure). Somehow, the presence of the non-toxic C-terminal domain WSCI contributed to enhance cytotoxicity of the bifunctional chimeric product.



Keywords: cell toxicity, ribosome inactivating proteins, protease inhibitors.

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